Incidence, Severity, and Characterization of *Phytophthora* Foot Rot of Citrus in Texas and Implications for Disease Management

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Abstract

Phytophthora-induced foot rot, also known as gummosis, is an important disease affecting citrus production worldwide. In Texas, the third-largest citrus-producing state in the United States, limited information is available on the etiology and epidemiology of foot rot in commercial orchards. This study comprises a survey of foot rot incidence and severity in Texas and the characterization of *Phytophthora* isolates associated with the disease. Surveys in 2015 and 2017 of 30 orchards in the Lower Rio Grande Valley (LRGV) region where commercial citrus production is concentrated in the state revealed that foot rot occurred in 97% of the orchards assessed. Overall, foot rot symptoms were observed on 33.7% of the trees evaluated and the disease severity index in the region was rated at 14.2 and 16.5% in 2015 and 2017, respectively. Lesions were mostly present on the scion, while the rootstock (sour orange) was not affected. *Phytophthora nicotianae* was the only *Phytophthora* sp. isolated from

the surveyed orchards and from five additional residential sites on the Texas Coastal Bend (TCB). Sporangia and chlamydospores from 34 representative LRGV isolates of *P. nicotianae* were larger than those of TCB isolates. In both LRGV and TCB, A1 and A2 mating types were present in the same location, albeit the A2 mating type was more prevalent. All isolates were sensitive to mefenoxam (50% inhibition in the presence of mefenoxam [EC₅₀] < 0.5 µg/ml), except for one TCB isolate (EC₅₀ = 143.6 µg/ml). Our research indicates that treatment for *Phytophthora* foot rot in the region is necessary and, although mefenoxam is still useful, alternating chemistries for resistance management are required.

Keywords: citrus foot rot, Lower Rio Grande Valley, mefenoxam, *Phytophthora nicotianae*

Phytophthora foot rot and root rot are important citrus diseases in all of the main citrus-producing regions in the world (Erwin and Ribeiro 1996). Under suitable conditions, *Phytophthora* spp. can infect the root system, causing root rot and the destruction of the feeder roots, which turn soft, with the cortex sloughing off from the stele, leading to poor water and nutrient uptake (Graham et al. 2014). Foot rot, also known as gummosis, is characterized by lesions that extend from the bud union to the major limbs of the scion and the production of amber-colored gum exudates in the branches (Graham and Feichtenberger 2015). On scions grafted to highly susceptible rootstocks such as sweet orange, Cleopatra mandarin, and rough lemon, lesions may extend around the base of the main trunk, causing partial or complete girdling and cambium damage. Affected trees show small chlorotic leaves, a thinning canopy, and twig dieback, leading to reduction of yield and fruit size (Graham and Feichtenberger 2015). *Phytophthora* brown rot of fruit causes the rind to become leathery and light brown in color. Infected fruit fall off the tree before harvest but some fruit may not show symptoms until during storage, causing major postharvest problems (Erwin and Ribeiro 1996).

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The three main *Phytophthora* spp. that have been identified to cause foot rot and root rot in citrus include *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur), *P. citrophthora* (R. E. Sm. & E. H. Sm) Leonian, and *P. palmivora* (E. J. Butler) E. J. Butler (Graham and Menge 1999). In subtropical climates where elevated temperatures and high humidity are common, *P. nicotianae* is the most prevalent species, whereas *P. citrophthora* is mostly found in Mediterranean climates (Graham and Menge 1999; Timmer et al. 2000). In Florida, the most common species isolated from soils in disease-affected orchards are *P. nicotianae* and *P. palmivora* (Graham et al. 1998; Zitko and Timmer 1994; Zitko et al. 1991). In California, *P. nicotianae* and *P. citrophthora* have been the main *Phytophthora* spp. isolated from the citrus rhizosphere, with *P. nicotianae* being more abundant in summer than in cooler months (Dirac et al. 2003; Menge et al. 1988). A recent report indicated that *P. citrophthora* and *P. syringae* are mostly responsible for causing brown rot of citrus in California, with *P. citrophthora* occurring year-round, and *P. syringae* present only in the winter months (Hao et al. 2018). In Texas, earlier studies reported the presence of *P. nicotianae* in bark, soil, and fruit collected from commercial citrus orchards (Timmer 1973). More recently, PCR-based approaches have identified the presence of *P. nicotianae* (Kunta et al. 2007; RoyChowdhury et al. 2016) and *P. citrophthora* (RoyChowdhury et al. 2016), although the latter species has not been confirmed by culturing.

Commercial citrus production in Texas is located in Hidalgo, Cameron, and Willacy Counties, a region called the Lower Rio Grande Valley (LRGV; Fig. 1). Foot rot has been reported in most commercial orchards since the introduction of citrus to this region (Timmer 1972). Flood irrigation is the prevalent irrigation method for citrus production. Highly calcareous soils, soil salinity (800 to 900 mg liter⁻¹), and poorly drained soils in the LRGV favor *Phytophthora* sp. establishment and continuous inoculum pressure (Blaker and MacDonald 1986; Timmer 1972; Wiedenfeld 2008).

A recent assessment of *Phytophthora* spp. soil propagule counts in 61 citrus orchards across the LRGV indicated that 33% of orchards had populations exceeding 10 CFU/cm³ (RoyChowdhury et al. 2016), the treatment threshold for this pathogen (Graham and Menge 1999). However, the current incidence and severity of foot rot in Texas citrus orchards is largely unknown.

Control strategies against foot rot and root rot of citrus include the use of resistant rootstocks, fungicide treatments, and proper water management (Hao et al. 2019). Sour orange is the most common rootstock used for citrus production in the LRGV due to its moderate tolerance to high soil pH and root rot. However, foot rot can develop on aerial parts of the tree on susceptible scion cultivars independently of the rootstock (Furr and Carpenter 1961; Timmer 1972). Metalaxyl, mefenoxam, and fosetyl-Al are systemic fungicides that have been widely used for controlling *Phytophthora* diseases on citrus and they are effective in improving root growth and fruit yield (Sandler et al. 1989). However, due to their excessive use, resistant isolates of *P. nicotianae* have been reported in citrus nurseries and young orchards in Florida (Timmer et al. 1998). In California, there are reports of *P. citrophthora* isolates resistant to potassium phosphite fungicides (Förster et al. 2016). In Texas, citrus growers also utilize these chemistries for managing *Phytophthora* diseases but there is no information available on the sensitivity of Texas isolates to these fungicides.

Heterothallic species such as *P. nicotianae* require both mating types (A1 and A2) to produce sexual spores (Judelson and Blanco 2005). Oospores can act as resting spores due to their thick walls that protect spore viability (Judelson and Blanco 2005). In the LRGV, the presence of the two mating types of *P. nicotianae* has been reported (Timmer 1973). The possible occurrence of oospores in citrus orchards suggests that the population may have high genetic and phenotypic diversity, which may have implications in pathogen adaptation to changing environmental conditions such as development of fungicide resistance.

In order to develop more effective strategies to manage *Phytophthora* foot rot in the LRGV region, the goals of this study were to (i) conduct surveys to quantify the incidence and severity of foot rot in commercial citrus orchards in the LRGV, (ii) identify the *Phytophthora* spp. associated with citrus foot rot and gummosis in the LRGV, (iii) characterize the isolates for phenotypic diversity

and pathogenicity, and (iv) evaluate their sensitivity to the commonly used fungicide mefenoxam.

Materials and Methods

Disease surveys. Surveys were conducted in commercial citrus orchards in the LRGV region (Hidalgo, Willacy, and Cameron Counties) from February to June 2015 and 2017. In all, 30 orchards were selected, reflecting the extent of citrus production in each county: 20 from Hidalgo, 8 from Cameron, and 2 from Willacy County. *Citrus* spp. included grapefruit (*Citrus × paradisi* Macf.; 23 orchards), sweet orange (*Citrus sinensis* (L.) Osbeck; five orchards), and tangerine (*C. reticulata* Blanco; two orchards), all grafted onto sour orange rootstock (*C. aurantium* L.). Orchards ranged in size from 4 to 8 ha. Twenty randomly selected trees along a diagonal transect in each orchard were evaluated for foot rot incidence and severity. Incidence was determined as a percentage of symptomatic trees of total number of trees assessed. Disease severity was assessed based on a scale of 0 to 5, where 0 = lesion-free tree; 1 = tree with <10% of the main trunk covered with lesions, callus formation, and no vertical lesion extension to the branches; 2 = tree with 11 to 35% of the main trunk covered with lesions, wound closed with some callus formation; 3 = tree with 65% of the main trunk covered with lesions, lesions vertically extended to the branches; 4 = tree with 90% of the main trunk covered with lesions, always associated with gummosis, lesions vertically extended to the branches; and 5 = tree with >90% of the trunk covered with lesions, showing extensive gummosis and extending to major branches. The disease rating scale was utilized to calculate the disease severity index (DI) per orchard using the formula $DI (\%) = [\sum(vf)/(ND)] \times 100$ (McKinney 1923), where v = foot rot severity score, f = frequency of trees with a given rating, N = total number of trees assessed in the orchard, and D is the highest disease severity score. The location of lesions below or above the bud union and their presence on the tree branches versus the trunk were noted. Orchard age was also recorded. Orchards ≥ 20 years of age were categorized as old and those <20 years old as young. To evaluate foot rot incidence and severity progression in flood-irrigated orchards (the prevalent irrigation method in the LRGV), 20 of the 30 orchards studied in 2015 were evaluated again in 2017.

Isolation and quantification of *Phytophthora* propagules. A soil core 20 cm in depth was collected from the drip line of each of

Fig. 1. Surveyed areas in the Texas Lower Rio Grande Valley and Texas Coastal Bend.

the 20 evaluated trees per orchard. A composite sample of the orchard was generated by mixing 100 cm³ of each of the 20 cores. Soil cores from five residential trees along the Texas Coastal Bend (TCB) were collected from the four quadrants within the drip line in each tree and mixed thoroughly to make one composite sample per site. *Phytophthora* spp. propagules were quantified from each composite sample as described previously (Timmer et al. 1988). Briefly, 100 cm³ of soil was saturated in 100 ml of sterile distilled water and allowed to drain for 48 h. A 10% (vol/vol) soil slurry was made by mixing 10 cm³ of drained soil in 90 ml of sterile distilled water. The slurry (1 ml) was plated onto each of five plates containing PARPH (5 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifamycin, 50 mg of pentachloronitrobenzene, and 50 mg of hymexazol per liter)—corn meal agar (CMA; Hardy Diagnostics, Santa Maria, CA, U.S.A.). The plates were incubated in the dark for 5 days at 23 ± 2°C, and distinctive *Phytophthora* colonies were quantified. Propagule counts were recorded as CFU per cubic centimeter of soil. Two to three *Phytophthora* colonies per location were isolated and subcultured to obtain single zoospore colonies as previously described (Erwin and Ribeiro 1996) to generate single-genotype isolates for identification and characterization.

***Phytophthora* sp. isolation from roots.** Fibrous roots from randomly selected soil samples were surface sterilized with 70% ethanol for 10 s, immersed in 0.6% sodium hypochlorite solution for 1 min, and rinsed with sterile water three to four times before plating onto PARPH-CMA. Plates were incubated in the dark for 5 days, and *Phytophthora* colonies were subcultured on 10% clarified V8 agar plates (CV8A) (Ferguson and Jeffers 1999). Single-zoospore colonies were obtained from all the *Phytophthora* isolates from roots, as previously described (Erwin and Ribeiro 1996).

Molecular identification. *Phytophthora* colonies growing in 10% clarified V8 (CV8) broth (100 ml of Campbell V8 vegetable juice, clarified by centrifugation at 8,000 rpm for 10 min, and 1 g of CaCO₃ per liter of water) at 22°C for 7 days were used to isolate total DNA with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia CA, U.S.A.) according to the manufacturer's instructions. The nuclear ribosomal DNA internal transcribed spacer (ITS) region, including the 5.8S and the mitochondrial cytochrome c oxidase (*cox*) genes, was amplified using the primer pair ITS4 and ITS6 and FMPhy-8b and FMPhy-10b, respectively (Cooke et al. 2000; Martin et al. 2004; White et al. 1990). The PCR mixtures consisted of 1× PCR buffer, 2 mM MgCl₂, 0.2 mM dNTP mix, 0.2 µM each forward and reverse primer, 1.25 U of GoTaq DNA polymerase (Promega Corp., Madison, WI, U.S.A.), and nuclease-free water to make a 50-µl total volume. Amplification was carried out in a MyCycler thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.), with an initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for ITS primers; or 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s for *cox* primers. PCR products were visualized by electrophoresis in a 1% agarose gel stained with ethidium bromide. The PCR products were purified using QIAquick PCR purification (Qiagen) and submitted to McLAB DNA Sequencing Services (San Francisco, CA, U.S.A.) for sequencing in both directions. The sequences were compared for similarities in the NCBI GenBank database using the BLASTn algorithm.

Cultural characterization of *Phytophthora* isolates. Morphological characterization was performed for selected isolates to represent most orchards. Sporangia formation was induced by incubating actively growing mycelium of a 7-day-old culture on 10% CV8A under fluorescent light for 24 h. Agar plugs were mounted on a slide, stained with 1 to 2 drops of 0.1% Lacto-Fuchsin (0.1 g of acid fuchsin per 100 ml of 85% lactic acid) and incubated overnight at 23 ± 2°C. Sporangia were visualized using ×40 magnification objective with a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany). The length and breadth of five sporangia per isolate were measured with the microscope image analysis software program (Leica application software; Leica Microsystems). Chlamydospores were observed in 10- to 15-day-old 10% CV8A cultures. The diameters of five chlamydospores per isolate were measured as above. The mating

type was identified by pairing isolates with known A1 and A2 tester isolates of *P. capsici* (provided by Dr. J. L. Hernandez, Centro de Biotecnología Genómica, IPN, Reynosa, Mexico). Plugs (5 mm in diameter) from unknown and tester isolates were placed on opposite sides of 10% CV8A plates (Erwin and Ribeiro 1996). Plates were incubated at 23 ± 2°C in the dark for 10 days. The presence of oospores in the interaction zone was recorded and used to determine the mating type. Agar plugs with oospores were mounted on a microscope slide and stained with Lacto-Fuchsin to measure antheridium width, as well as oogonium and oospore diameters. Colony patterns were determined for 7-day-old colonies on potato dextrose agar (PDA) and CV8A and classified as slight chrysanthemum, slight stellate, slight rosaceous to diffuse, radiate, or chrysanthemum (Erwin and Ribeiro 1996).

Sensitivity to mefenoxam. Plugs (5 mm in diameter) from actively growing 5-day-old *Phytophthora* spp. cultures were transferred onto 10% CV8A amended with mefenoxam (Ridomil Gold SL EC; Syngenta Crop Protection, Greensboro, NC, U.S.A.) active ingredient at 0, 0.1, 1, or 10 µg/ml. All isolates were plated in triplicate and incubated in the dark for 5 days at 23 ± 2°C. Radial growth was measured and averaged, and percent growth was calculated by dividing the radial growth on amended media by growth on non-amended media (Timmer et al. 1998). Two independent experiments were performed, and data were pooled for analysis after confirming homogeneity of variances using Bartlett's test. Percent growth inhibition was determined and converted to probits (MSEXCEL formula NORM.S.INV [probability]). Probit values were plotted against log₁₀-transformed fungicide concentrations (Finney 1962). The regression equation was used to calculate 50 and 90% inhibition in the presence of mefenoxam (EC₅₀ and EC₉₀, respectively) values. Isolates that grew with mefenoxam at 10 µg/ml were also tested at 100 and 1,000 µg/ml.

Pathogenicity tests on citrus and alternative hosts. The pathogenicity of 20 selected isolates was tested on seedlings of sour orange (*C. aurantium*) and four noncitrus hosts, including tomato (*Solanum lycopersicum*), garden bean (*Phaseolus vulgaris*), summer squash (*Cucurbita pepo*), and Russell hybrid lupin (*Lupinus polyphyllus*). Zoospore inoculum was prepared by transferring 5-mm-diameter plugs from 5-day-old cultures into 15 ml of a sterile soil solution (Jeffers 2006). After 24 h under fluorescent light, zoospore release was initiated by incubating at 4°C for 3 h and then in the dark for 30 min at 23 ± 2°C. Roots of three 6-week-old plants grown under greenhouse conditions were immersed overnight in a zoospore suspension (10⁴ zoospores/ml) of each tested isolate (Matheron and Matejka 1990). Control plants were inoculated in sterile distilled water. Seedlings were planted into sterile potting soil mix, maintained in the greenhouse, and watered daily to maintain high moisture conditions. Pathogenicity on sour orange seedlings was assessed on nine seedlings per isolate after 10 weeks. For each isolate, 30 root segments with visible lesions were plated onto PARPH-CMA. Plates were incubated in the dark for 4 to 5 days, and the percentage of root segments that resulted in *Phytophthora* colonies was calculated.

On alternative hosts, plant mortality was assessed after 3 days, and the experiment was terminated after 7 days. Seedling mortality and the presence of lesions on roots, stems, and leaves were recorded. The entire seedlings, including roots, stems, and leaves, were cut into segments, plated onto PARPH-CMA, and incubated in the dark for 4 to 5 days. Based on lesion size, percent plant part infected, and mortality, isolates were grouped into three categories: highly virulent (+++) on the host if all three seedlings died, the lesions covered more than 90% of the seedling, and *Phytophthora* sp. was isolated from all plated plant segments; moderately virulent (++), if two of the three seedlings died, lesions were present in 40 to 90% of the seedling, and all plated segments resulted in positive isolations; and low virulence (+), when only one of the three seedlings died, lesions were present in less than 40% of the seedling, and *Phytophthora* sp. was recovered from the plated plant segments. Isolates were considered nonpathogenic (negative) if they caused no mortality, no lesions were present, and no *Phytophthora* sp. grew from plated segments.

Statistical analysis. Percent data were arcsine-transformed while propagule counts were $\log(x + 1)$ -transformed before analyses. Analysis of variance was used to evaluate the effect of tree age on disease incidence and severity in the orchards and the number of propagules. Data were analyzed with PROC GLM (SAS version 9.4 for Windows; SAS Institute, Cary, NC, U.S.A.) to test significance. Mean separations were made by the Student-Newman-Keuls multiple range test if F values were significant ($P \leq 0.05$).

Results

Foot rot incidence and severity. A 2015 survey in the three commercial citrus-growing counties in the LRGV of Texas revealed that foot rot was present in 97% of the 30 orchards surveyed. The incidence within orchards ranged from 5 to 90% and averaged 30, 33.6, and 40% in Cameron ($n = 8$), Hidalgo ($n = 20$), and Willacy ($n = 2$) Counties, respectively. Overall, 33.7% of the 600 surveyed trees showed symptoms. Foot rot severity ranged from 1 to 57%, with an average of 14.2% (Table 1).

Similar results were obtained in the 2017 survey. Mean foot rot incidences in orchards were 39 and 30.6% in Cameron and Hidalgo Counties, respectively. Although a slight increase in disease severity was observed in Cameron County, no notable change was observed in Hidalgo County (Table 1).

During the 2015 sampling, *Phytophthora* spp. propagules in orchard soils ranged from 0 to 118 CFU/cm³, and 23 of the 30 orchard soils had more than 10 CFU/cm³. Propagule counts in the 2017 assessment were significantly lower than in 2015 ($P = 0.0094$), with a mean of 9.1 CFU/cm³ and ranged from 0 to 46 CFU/cm³, and 8 of the 20 orchards had more than 10 CFU/cm³ (Table 2).

Older (>20 years) grapefruit orchards had significantly higher foot rot incidence ($P = 0.01$) and disease severity ($P = 0.02$) compared with younger (<20 years) orchards (Fig. 2). *Phytophthora* spp. soil propagule counts did not differ significantly between older and younger orchards. Symptoms of foot rot were present on 54% (214 of 400) of tree branches compared with 34% (135 of 400) of tree trunks evaluated. There were no significant differences in the incidence of symptoms on trunk and branches between grapefruit and sweet orange trees ($P = 0.9$ and 0.5, respectively) (Table 3). Only 7.25% (29 of 400) of the trees exhibited symptoms of foot rot on the sour orange rootstock.

Pathogen isolation and identification. During the 2015 survey, in total, 93 isolates were obtained from citrus roots and soil samples. In all, 81 isolates originated from 27 citrus orchards in the LRGV and 12 isolates from soils from residential citrus trees in the TCB. ITS sequence analysis of 89 isolates indicated 97 to 100% sequence identity to *P. nicotianae*, including all isolates collected from the TCB. Four isolates were identified as *Pythium ultimum* (PYE2 & PYE3), *P. nodosum* (PYE1), and *Phytophthora vexans* (PyMi5) (Supplementary Table S1). Sequencing of the *cox* gene of 47 isolates confirmed the identity of isolates as *Phytophthora nicotianae*.

The assembled sequences were deposited into the GenBank database (accession numbers MH290367 to MH290397, MH290399 to MH290417, MH290419 to MH290446, and MH341608 to MH341622) (Supplementary Table S1). None of the sequenced isolates showed matches to *P. citrophthora*.

Morphological characterization. In all, 34 (LRGV = 29 and TCB = 5) of the 89 *Phytophthora* isolates identified as *P. nicotianae* were selected for morphological characterization. Colony morphology on 10% CV8A varied from chrysanthemum, radiate, stellate, to nondefined, whereas isolates grown on PDA exhibited mostly

Table 2. *Phytophthora* spp. soil propagules in surveyed orchards in 2015 and 2017 in the Lower Rio Grande Valley of Texas

County	N ^y	2015	N ^y	2017
		Mean soil propagules (range)		Mean soil propagules (range)
Cameron	8	21.1 (0–62)	5	5.8 (0–13)
Hidalgo	20	31.5 (0–118)	15	12.9 (0–46)
Willacy	2	9.5 (4–15)	0	...
Mean ^z	...	26.5 ± 5.6		9.1 ± 2.3
P value	...	0.9		0.6

^y N = number of orchards.

^z Mean (range in parentheses) was significantly different between years 2015 and 2017 ($P = 0.0094$).

Fig. 2. Effect of orchard age on incidence and severity of *Phytophthora* foot rot of citrus. Orchards more than 20 years of age ($n = 17$) were compared with those less than 20 years old ($n = 9$). Error bars indicate standard error and different letters indicate significant differences ($P < 0.05$).

Table 3. Incidence of trees showing foot rot symptoms on branches, trunks and rootstocks^y

Scion	Orchards/trees ^z	Infection (%) of		
		Branch	Trunk	Rootstock
Grapefruit	15/300	56.3 (10–90)	33.3 (10–70)	7.6 (0–30)
Sweet orange	3/60	51.6 (30–85)	30 (25–35)	5 (0–15)
Tangerine	2/40	35 (15–55)	32.5 (20–45)	7.5 (0–15)
P value	...	0.5	0.9	0.8

^y Incidence of branch infections was significantly higher than main trunk infections ($P = 0.006$) (range in parentheses).

^z Number of orchards/trees assessed.

Table 1. *Phytophthora* foot rot incidence and severity in the citrus producing counties of the Lower Rio Grande Valley of Texas during spring of 2015 and 2017 survey

County	N ^x	2015		N ^x	2017	
		Incidence (%) ^y	Severity (%) ^z		Incidence (%) ^y	Severity (%) ^z
Cameron	8	30.0 (10–90)	10.37 (2–20)	5	39.0 (2–42)	20.4 (10–70)
Hidalgo	20	33.6 (5–80)	15.6 (1–57)	15	30.6 (5–35)	15.2 (15–55)
Willacy	2	40.0 (25–55)	15.0 (10–20)	0	...	...
Means	...	33.7 ± 4.2	14.2 ± 2.2	...	32.75 ± 4.3	16.5 ± 3.0

^x Number of orchards.

^y Mean incidence was determined as percentage of trees expressing symptoms of the total number of trees assessed (range in parentheses).

^z Mean severity was calculated from the disease severity indexes from each orchard assessed per county (range in parentheses).

stoloniferous growth (Supplementary Table S2). Vegetative hyphae were coralloid, showing hyphal swellings. More than one type of sporangium shape was noted for the same isolates. Ovoid, obpyriform, and spherical with single papilla were the more common shapes but spherical, bipapillate, and turbinate sporangia were also present. Both terminal and intercalary chlamydospores were observed (data not shown).

Sporangial size varied considerably among isolates; sporangium length and breadth ranged from 23.0 ± 1.2 to 47.5 ± 2.5 μm (mean = 47.7 μm) $\times$ 28.0 ± 1.2 to 65.7 ± 3.3 μm (mean = 36 μm), respectively. Mean chlamydospore diameters for individual isolates ranged from 23.0 ± 1.2 to 50.7 ± 1.9 μm , with a mean for all isolates of 36.1 μm (Supplementary Table S2). Spore measurements conformed to the reported dimensions of *P. nicotianae* type species (Erwin and Ribeiro 1996). When LRGV and TCB isolates were compared, significant differences in chlamydospore diameter and sporangial length and width were detected (Table 4). Isolates obtained from sites along the TCB had significantly smaller sporangia lengths and breadths as well as chlamydospore diameters than those from the LRGV sites, although the sporangia length/breadth ratio was similar.

Mating type and characteristics of oospores. In total, 25 isolates were of mating type A2 and 9 isolates were of A1, because they only produced sexual spores when they were paired with A1 and A2 *P. capsici* tester isolates, respectively. Both mating types were present at the same site in 2 of the 20 LRGV orchards and 2 of the 5 TCB sites. Smooth and spherical amphigynous antheridia were formed in all pairings. The oogonium diameter ranged from 22.6 ± 1 to 26.8 ± 0.4 μm , with an average of 24.9 ± 0.16 μm . The antheridium diameter ranged from 8.0 ± 1.2 to 13.4 ± 0.6 μm . Oospores were plerotic, ranging from 17.5 ± 0.5 to 22.8 ± 0.4 μm in diameter. Sizes of sexual reproductive structures of isolates from the LRGV and TCB were not significantly different ($P > 0.05$) (Table 4; Supplementary Table S3). None of the isolates produced sexual reproductive structures in single cultures, indicating that they were not self-fertile.

Mefenoxam sensitivity. *P. nicotianae* isolates from the LRGV exhibited different degrees of sensitivity to mefenoxam. Five isolates were highly sensitive, with EC_{50} values for mycelial growth of 0.001 $\mu\text{g/ml}$, and 21 isolates had EC_{50} values between 0.01 and 0.2 $\mu\text{g/ml}$. Three isolates had EC_{50} values between 0.2 and 0.5 $\mu\text{g/ml}$ and were rated as moderately resistant because they had EC_{90} values between 3.0 and 10 $\mu\text{g/ml}$ (Table 5; Supplementary Table S4).

Among the five isolates from the TCB area, one was highly sensitive, three were sensitive, and one was resistant. Growth of the resistant isolate (PhCC63) at 100 $\mu\text{g/ml}$ was similar as on nonamended agar. EC_{50} and EC_{90} values for this isolate were 143.6 and $2,615$ $\mu\text{g/ml}$, respectively (Table 5; Supplementary Table S4).

Pathogenicity tests on citrus and noncitrus hosts. Pathogenicity of the isolates tested varied significantly, because not all of them were able to cause infection of every host. Sour orange seedlings inoculated with 15 *P. nicotianae* isolates did not show visible signs of wilting or yellowing of leaves. However, 11 of the isolates caused some symptoms on the main root, while 4 were not pathogenic (Table 6).

The alternative host *Lupinus* was highly susceptible, and more than 88% of the plants were dead 4 days after inoculation. Lesions were present on all parts of the plant, and the pathogen was successfully isolated from the seedling when plated on selective media.

The numbers of *P. nicotianae* isolates pathogenic on squash and tomato seedlings were equal ($n = 16$); however, a higher mortality rate was observed on tomato because 12 isolates caused death of all inoculated seedlings, in contrast to only 4 isolates on squash. Only 7 of the isolates tested were able to colonize bean. They were recovered from stems and roots, although no visible lesions were observed. Only one isolate (PhLF25) was pathogenic on all hosts, whereas one isolate (PhE32) did not cause disease on any of the four noncitrus hosts and only caused root lesions on sour orange (Table 6).

Discussion

This study is the first comprehensive report on the incidence and severity of *Phytophthora* foot rot of citrus in the LRGV of Texas. Our work demonstrated that foot rot is widespread in the main citrus-growing region of Texas, occurring in all commercial citrus orchards evaluated at various incidence levels. Although our survey indicated a mean severity index of 16.5, this may be underestimating the foot rot severity in the region because dead trees were not considered, as the cause of tree mortality was not known. The results from this study warrant the investigation of the economic impact of *Phytophthora* foot rot from tree death and decline in productivity.

Another important finding from this work is that lesions were mostly present on the scion and not on the rootstock. Although sour orange is considered a tolerant rootstock, it is surprising that, with flood irrigation, clay soils, and poor drainage, the percentage of foot rot on the rootstock was very low in surveyed orchards; mostly because during flood irrigation the bud union can be covered in orchards where trees are budded below 30 cm. The higher percentage of trees with lesions in branches and limbs compared with the rootstock is similar to what has been observed in Spanish orchards, with 92% of lesions on the scion (Alvarez et al. 2008). Infection of scions could have occurred when *P. nicotianae* propagules were splashed onto wounded branches and trunks, especially those located close to the ground (Graham and Menge 1999). Hedging and topping citrus for tree size management create wounds that could serve as entry points for *Phytophthora* spp. inoculum. If equipment becomes contaminated, it could spread the pathogen not just from tree to tree but also among orchards if not decontaminated properly. High incidence of foot rot and gummosis has been reported from other citrus-growing regions of the world such as Kenya, where noncertified planting material is used and favorable conditions for disease development are present (Mounde et al. 2009). In Texas, the mandatory use of potted citrus trees from certified nurseries did not come into effect until 2013 (Da Graça and Van Ness 2019). Future studies will

Table 5. Mefenoxam sensitivity of *Phytophthora nicotianae* isolates from citrus orchards in the Lower Rio Grande Valley (LRGV) and from the Texas Coastal Bend (TCB)

Sensitivity rating	Number of isolates (LRGV/TCB)	EC_{50} value ^z
Highly sensitive	5/1	<0.001 $\mu\text{g/ml}$
Sensitive	21/3	0.01 to 0.2 $\mu\text{g/ml}$
Moderately resistant	3/0	0.2 to <1 $\mu\text{g/ml}$
Insensitive	0/1	>1 $\mu\text{g/ml}$

^z EC_{50} = 50% inhibition in the presence of mefenoxam.

Table 4. Morphological characteristics of isolates of *Phytophthora nicotianae* from sites in the Lower Rio Grande Valley (LRGV) and the Texas Coastal Bend (TCB)^y

Site (<i>n</i>) ^z	Chlamydospore diameter (μm)	Sporangium length (L) (μm)	Sporangium breadth (B) (μm)	LB ratio	Oogonium diameter (μm)	Oospore diameter (μm)	Antheridium diameter (μm)
LRGV (29)	36.5 ± 0.38 a	42.28 ± 0.42 a	36.4 ± 0.3 a	1.32 ± 0.01	24.9 ± 0.16	20.3 ± 0.18	11.4 ± 0.14
TCB (5)	28.1 ± 0.63 b	38.4 ± 0.81 b	29.1 ± 0.58 b	1.30 ± 0.01	24.5 ± 0.56	20.3 ± 0.42	12.2 ± 0.46
<i>F</i> value	15.08	36.36	40.17	...	1.55	0.1	3.73
<i>P</i> value	0.0001	0.0001	0.0001	...	0.21	0.9	0.07

^y Means followed by different letters are significant at $P < 0.05$.

^z Site (number of isolates).

reveal whether foot rot incidence is reduced in new orchards planted with certified materials.

This study showed that the pathogen associated with this disease in Texas is *P. nicotianae*, because it has been the only *Phytophthora* sp. isolated from citrus soils in this work and in previous studies (Kunta et al. 2007; Timmer 1973). *P. nicotianae* is known to have a higher optimum temperature for growth, which may provide an advantage over other *Phytophthora* spp. that cause diseases on citrus and other subtropical crops (Panabieres et al. 2016). The predominance of *P. nicotianae* in Texas soils could be due to the high temperatures reached during the summer months. However, in other regions with warmer climates such as India or Florida, foot rot and root rot infections are caused by *P. nicotianae* and *P. palmivora* (Graham et al. 1998; Sonavane and Venkataravanappa 2017). In Mediterranean climates such as Spain and the Western Cape region of South Africa, *P. citrophthora* is the most prevalent species and *P. nicotianae* populations are less abundant (Alvarez et al. 2008; Meitz-Hopkins et al. 2014). In other regions, seasonal fluctuations affect the ratio between *Phytophthora* spp. present in the same location. In California, root rot is caused by *P. nicotianae* during warmer times of the year, whereas *P. citrophthora* is present year-round (Hao et al. 2018). Although *P. citrophthora* was detected by PCR in Texas soils in 2016 (RoyChowdhury et al. 2016), this species was not detected in our surveys. If *P. citrophthora* is present, its abundance is below the detection limit of our culturing assay and may not have an impact in the development of foot rot of citrus in the state.

Characterization of *P. nicotianae* isolates collected from the LRGV and the TCB showed great diversity in chlamydospore and sporangium size and fungicide sensitivity, as well as virulence and pathogenicity to citrus and noncitrus hosts. The finding that these propagules were larger in commercial orchards in the LRGV compared with residential areas in the TCB may imply some adaptation to the environment such as different soil conditions and management practices. Flood irrigation of commercial orchards in Texas may produce anaerobic conditions alternating with very dry soil conditions (Davidson and Janssens 2006). Although a trade-off between size and spore number is expected, larger chlamydospores may be more suited to withstand those extreme environments because larger size may increase spore viability (Mariette et al. 2018).

P. nicotianae has a wide host range (Erwin and Ribeiro 1996). However, in the present study, we have shown some degree of host specificity, because one isolate was nonpathogenic to any of the non-citrus hosts tested and others showed pathogenicity to some hosts but not others. We did not observe strong virulence on bean seedlings, although we were able to recover the pathogen from stem tissues. This is in accordance with other studies where *P. nicotianae* isolates were not pathogenic to snap bean seedlings and weakly virulent on pods (Olson et al. 2016). Sour orange seedlings showed tolerance to *Phytophthora* sp. because none of the isolates killed the seedlings, although they were able to colonize the roots. Continuous use of predominantly sour orange rootstock in LRGV is justified due to its desirable characteristics of tolerance to the pathogen and good performance in calcareous soils with poor drainage and salinity issues (Roose 2014).

Mefenoxam-based fungicides are widely used to control oomycete pathogens. Their extensive use in agriculture has led to the emergence of insensitive pathogen populations. In open-field nurseries and greenhouses, mefenoxam-resistant *P. nicotianae* isolates have been detected in Florida, North Carolina, and Virginia (Hu et al. 2008; Hwang and Benson 2005; Timmer et al. 1998). Although mefenoxam-resistant isolates were not found in commercial orchards of LRGV, some isolates displayed reduced sensitivity, which may indicate a transitioning stage for resistance development (Venkataramana et al. 2010). Because mefenoxam-insensitive isolates can be as aggressive as sensitive isolates with or without mefenoxam selection pressure (Café-Filho and Ristaino 2008; Chapara et al. 2011; Hu et al. 2008; Taylor et al. 2006; Timmer et al. 1998), a survey for resistance in citrus orchards with known history of mefenoxam use would be beneficial. Nonetheless, the absence of resistant isolates from the sampled LRGV orchards indicates that mefenoxam can still be an effective control for *Phytophthora* diseases in the area and that alternating fungicides such as oxathiapiprolin, fosetyl-Al, and phosphites, among others, as a resistance management strategy is required.

As management practices begin to change in the LRGV to address water shortages and threatening diseases such as huanglongbing, the study of *Phytophthora* diseases will also need to continue. New high-density plantings, implementation of raised beds and ground covers, and the increased use of water-saving irrigation methods (drip and

Table 6. Virulence and pathogenicity of *Phytophthora nicotianae* isolates and the tissues of pathogen recovery in citrus and noncitrus hosts^y

Isolate ID ^a	Sour orange	Lupinus	Tomato	Squash	Bean
LRGV					
PhC65	Negative	Stem, root, leaf+++	Stem, root, leaf+++	Root, stem+++	Root+
PhH66	Negative	Stem, root, leaf+++	Stem, root, leaf+++	Root, stem++	Na
PhLF2	Root+	Stem, root, leaf+++	Negative	Stem, root++	Negative
PhLF25	Root+	Stem, root, leaf+++	Root, stem+++	Root, stem++	Stem, root+
PhLF60	Negative	Na	Stem, root++	Negative	Negative
PhLF75	Root+	Stem, root, leaf+++	Negative	Root, stem+	Stem+
PhSB14	Na	Stem, root, leaf+++	Stem, root, leaf+++	Root, stem+++	Negative
PhA21	Na	Stem, root, leaf+++	Stem, root+++	Root, stem+++	Stem, root+
PhD4	Root+	Stem, root, leaf+++	Stem+++	Stem, root+	Root+
PhD6	Root+	Root+++	Stem, root+++	Stem, root+	Negative
PhE32	Root+	Negative	Negative	Negative	Negative
PhMC28	Root+	Stem, root, leaf+++	Stem, root, leaf+++	Root, stem+	Negative
PhMC56	Root+	Stem, root, leaf+++	Stem, root+++	Negative	Negative
PhMC72	Negative	Stem, root, leaf+++	Stem, root, leaf+++	Root, stem++	Stem+
PhMI81	Root+	Stem, root, leaf+++	Na	Root, stem++	Na
PhSJ23	Root+	Stem, root, leaf+++	Stem, root+++	Root, stem+++	Negative
PhW13	Na	Stem, root, leaf+++	Stem, root++	Root, stem++	Negative
TCB					
PhCC63	Na	Stem, root+	Stem+	Na	Negative
PhO44	Na	Stem, root, leaf+++	Stem, root, leaf+++	Stem, root+	Stem, root+
PhRP24	Root+	Root, stem++	Root, stem, leaf+	Root, stem++	Negative

^y Virulence was based on percent mortality and percent of plant segments from which *P. nicotianae* was recovered. Isolates were rated as +++ = highly virulent, ++ = moderately virulent, or + = weakly virulent; negative means nonpathogenic and Na = not assessed.

^z Regions in Texas: LRGV = Lower Rio Grande Valley and TCB = Texas Coastal Bend.

microsprinkler) will likely affect the incidence and severity of *Phytophthora* diseases. Therefore, further studies are required to evaluate the effect of these new orchard planting strategies.

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